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Initial Testing of a Novel Urine Culture Device

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The Diaslide urine culture device consists of a hinged case containing two opposing agar media separated by a sampler with a handle at one end and two bent sampler tips at the opposite end. The tips of the sampler are first dipped into the urine. The sampler is then pulled out through the casing, simultaneously inoculating both agar surfaces with a streaking dilution. As a result, individual colonies can be observed even when bacterial concentrations exceed 10^6 CFU/ml. The number of colonies on the Diaslide correlated linearly with CFU per milliliter as determined by dilution plating. The clinical performance of the Diaslide was compared with those of ordinary dipslides and conventional cultures with a sample of 473 prescreened hospital urine specimens. The sensitivity, specificity, and positive predictive value of Diaslide versus those of culture at the 10^4-CFU/ml cutoff level were 97.5, 98.3, and 98.3%, respectively, compared with 98.8, 95.7, and 97.2%, respectively, for dipslide versus culture. Similar results were found at the 10^5-CFU/ml cutoff level. Only 5.5% of the Diaslides required subculturing, compared with 14.7 and 9.4% of the dipslides and conventional cultures, respectively. The Diaslide proved more convenient than an ordinary dipslide for sampling low volumes of urine. These data suggest that the Diaslide is a simple, effective device for culturing of urine specimens.

Urine is one of the few biological specimens submitted to the clinical laboratory that require analysis of both the microbial species and the microbial concentration (9). Quantitative, conventional culture often employs a streaking dilution of the urine sample on solid media with a calibrated loop. This method enables appraisal of the microbial concentration in the sample and a presumptive initial identification, and it provides individual colonies for further evaluation (e.g., antibiotic susceptibility studies). One major disadvantage of this approach is that the urine sample itself must be transported to the microbiology laboratory, where it is then plated out by skilled personnel. Since urine is an excellent medium for bacterial culture (1), the number of bacteria present may increase rapidly in the interim. Once the urine is applied to a solid (e.g., agar) surface, however,

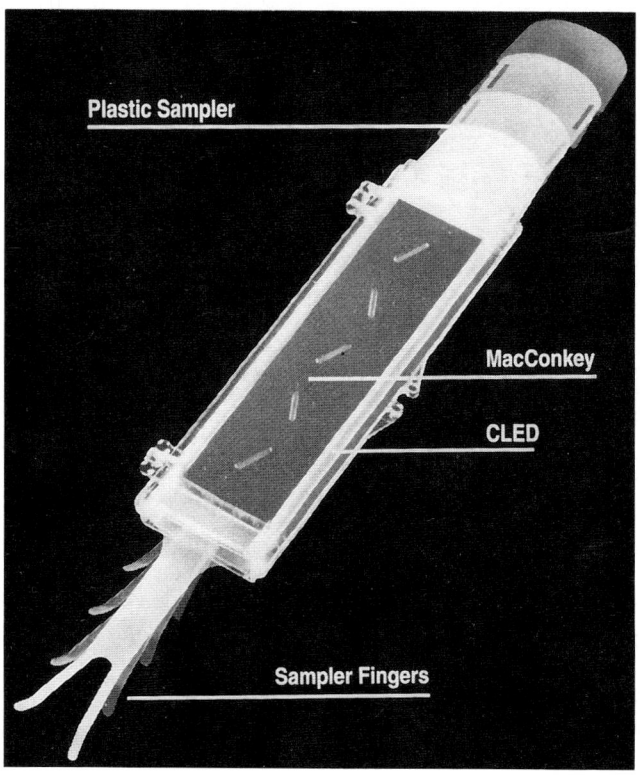

FIG. 1. Design of the Diaslide.

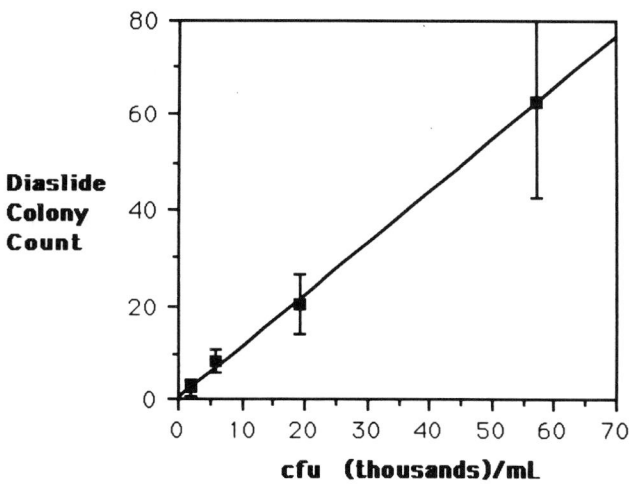

FIG. 2. Correlation between numbers of colonies on the Diaslide and CFU per milliliter in seeded urine samples. Urine, seeded with different numbers of *E. coli* cells, was sampled by using Diaslides (n = 20 for each bacterial concentration). The average Diaslide counts ± standard deviation (CLED) are plotted as a function of CFU per milliliter. Similar correlations (not shown) between enumeration of colonies on the MacConkey surface of the Diaslide and that by dilution plating were found.

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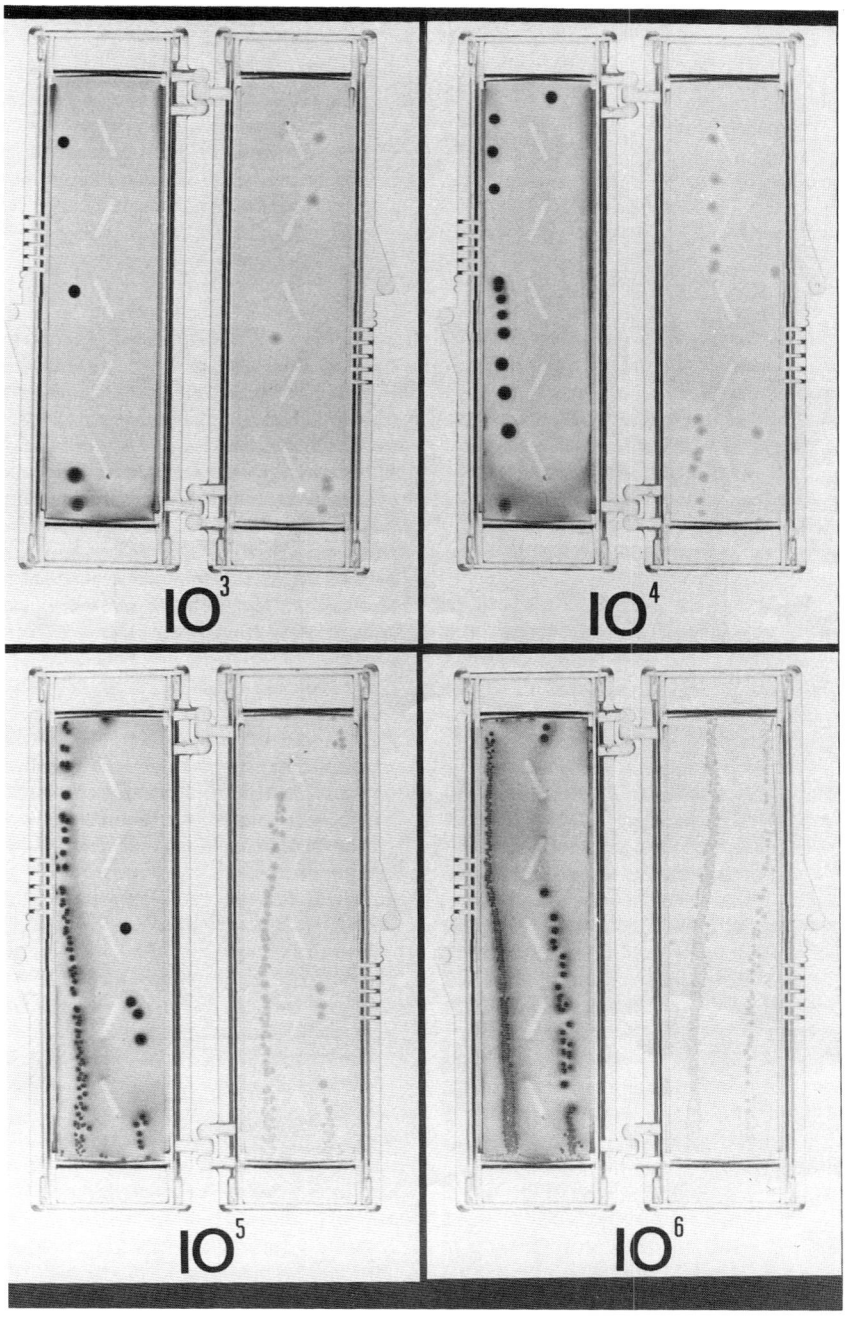

FIG. 3. Diaslide reference chart showing representative results ranging from 10^3 to 10^6 CFU/ml.

each multiplying bacterium yields only a single colony on subsequent incubation.

The requirement for a simple method of applying urine to solid surfaces at the collection site has yielded a variety of approaches, including filter paper strips (12), agar-coated pipettes (10), spoons (13, 14), glass slides (4), and, ultimately, plastic commercial "dipslides." The urine dipslide, with agar medium on each side of an immersible plastic paddle, usually employs a nonselective, electrolyte-deficient medium enriched with cystine (CLED) (13) and a medium selective for gram-negative bacteria (MacConkey or eosin-methylene blue). The dipslide has been shown previously to be a cost-effective, simple device for the detection of bacteriuria in both inpatient and outpatient settings, providing results comparable to those obtained by standard plate-streaking methods (5–8, 11, 15, 16, 18–20).

The conventional urine dipslide has several disadvantages which compromise its usefulness: (i) at concentrations in urine of $\geq 10^6$ CFU/ml, confluent growth is often obtained, complicating detection and subculturing, (ii) sampling of low volumes of urine with the dipslide is cumbersome, (iii) since a large amount of urine is absorbed by the immersed agar surfaces, there is a possibility of false-negative results due to carryover of inhibitory agents present in the urine, and (iv) the condensation which forms on the outer vial during incubation hinders examination and necessitates unscrewing

TABLE 1. Comparison of Diaslide and conventional culture

Cutoff (CFU/ml) and result by Diaslide[a]	No. of the following result by conventional culture[b]			Total
	Positive	Negative	Doubtful	
10^{4c}				
Positive	238	4	12	254
Negative	4	157	43	204
Doubtful	1	3	11	15
Total	243	164	66	473
10^{5d}				
Positive	206	5	3	214
Negative	4	226	5	235
Doubtful	2	5	17	24
Total	212	236	25	473

[a] Based on comparison with those of the reference chart in Fig. 2.
[b] Based on enumeration of colonies on plates.
[c] At a cutoff of 10^4, culture and Diaslide positives are those judged as $\geq 10^4$ CFU/ml; those judged as $\leq 10^3$ CFU/ml are considered negative, and those between 10^3 and 10^4 CFU/ml are recorded as doubtful.
[d] At a cutoff of 10^5, culture and Diaslide positives are those judged as $\geq 10^5$ CFU/ml; those judged as $\leq 10^4$ are considered negative, and those between 10^4 and 10^5 are recorded as doubtful.

of the cap and removal of the attached slide. Since the majority of urine cultures are negative, this compromises the device's user-friendliness and lengthens the processing time.

The purpose of the present investigation was to develop and test a novel device (17) which combines the advantages of both conventional culture and dipslides. Its effectiveness was evaluated by using reconstituted urine specimens and tested in a clinical trial to compare it with the effectiveness of conventional dipslides and culturing.

TABLE 2. Comparison of Diaslide with dipslide

Cutoff (CFU/ml) and result by Diaslide[a]	No. of the following result by dipslide[b]			Total
	Positive	Negative	Doubtful	
10^{4c}				
Positive	250	2	2	254
Negative	5	191	8	204
Doubtful	3	1	11	15
Total	258	194	21	473
10^{5d}				
Positive	213	1	0	214
Negative	6	229	0	235
Doubtful	3	7	14	24
Total	222	237	14	473

[a] Based on comparison with those in the reference chart in Fig. 2.
[b] Based on comparison with those in the manufacturer's reference chart.
[c] At a cutoff of 10^4, dipslide and Diaslide positives are those judged as $\geq 10^4$ CFU/ml; those judged as $\leq 10^3$ CFU/ml are considered negative, and those between 10^3 and 10^4 CFU/ml are recorded as doubtful.
[d] At a cutoff of 10^5, dipslide and Diaslide positives are those judged as $\geq 10^5$ CFU/ml; those judged as $\leq 10^4$ are considered negative, and those between 10^4 and 10^5 are recorded as doubtful.

TABLE 3. Summary of statistical data[a]

Cutoff (CFU/ml) and technique	Result (%) compared with that by Diaslide				
	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Total agreement[b]
10^4					
Culture	98.3	97.5	98.3	97.5	86
Dipslide	97.8	99.6	99.2	97.4	95.6
10^5					
Culture	98.1	97.9	98.1	97.8	94.4
Dipslide	97.3	99.6	97.3	99.6	96.4

[a] Sensitivities, specificities, positive predictive values, and negative predictive values were calculated exclusive of doubtful results.
[b] Total percent agreement was calculated as (number of true positives + number of true negatives + number of true doubtfuls) · 100/total.

MATERIALS AND METHODS

Description of the Diaslide. A schematic diagram of the Diaslide and its component parts is shown in Fig. 1. The casing consists of two hinged plastic sections, each containing agar medium (CLED and MacConkey) and folded with the two agar surfaces facing each other. A specially designed plastic inoculator (sampler) lies between the agar surfaces. At the V-shaped sampling end of the inoculator are two bent tips ("fingers") which are dipped into the urine sample. The sampler is then pulled out through the device, effecting simultaneously the inoculation and dilution streaking of both agars. Each agar surface is thus streaked by the tip of one of the tips, as well as by the bent "joint" of the other tip. The tip inoculation yields a streaking dilution of several orders of magnitude, whereas inoculation by the joint yields a relatively uniform spreading of the sample. Following inoculation, the sampler is discarded and the case is placed in a specially designed tray for upright incubation. Subsequent growth can be observed directly without opening the device. For subculturing of positive samples, the hinged casing is opened.

Microbial strains and growth conditions. *Escherichia coli* ATCC 25922 was maintained on brain heart infusion plates and inoculated into brain heart infusion broth (Difco Laboratories, Detroit, Mich.). Following overnight growth with shaking at 30°C, bacterial suspensions were frozen at −70°C in 3-ml aliquots. Prior to the experiments, aliquots were allowed to equilibrate to room temperature and serial dilutions were performed with fresh sterile urine to yield final concentrations between approximately 10^3 and 10^7 CFU/ml. For viable counts, 10-μl aliquots (at least 10 samples for each concentration) of the appropriate seeded urine samples were applied to brain heart infusion agar plates, and colonies were enumerated. Dipslides (Diagnostic Pasteur, Mames-la-Coquette, France) were inoculated by being immersed in seeded urine samples. Diaslides were inoculated by dipping the tips of the samplers approximately 2 cm into the urine samples and then drawing each sampler out through the casing.

In order to test the effect of an antibiotic on results, fresh urine was seeded with *E. coli* to a concentration of approximately 10^5 CFU/ml and ampicillin (Sigma, St. Louis, Mo.) was added to a concentration of 2 mg/ml. Dipslides (Pasteur) and Diaslides were inoculated with this suspension, and the results were compared with results for control urine containing the same bacterial concentration in the absence of an antibiotic.

Clinical experiments. An initial clinical evaluation was

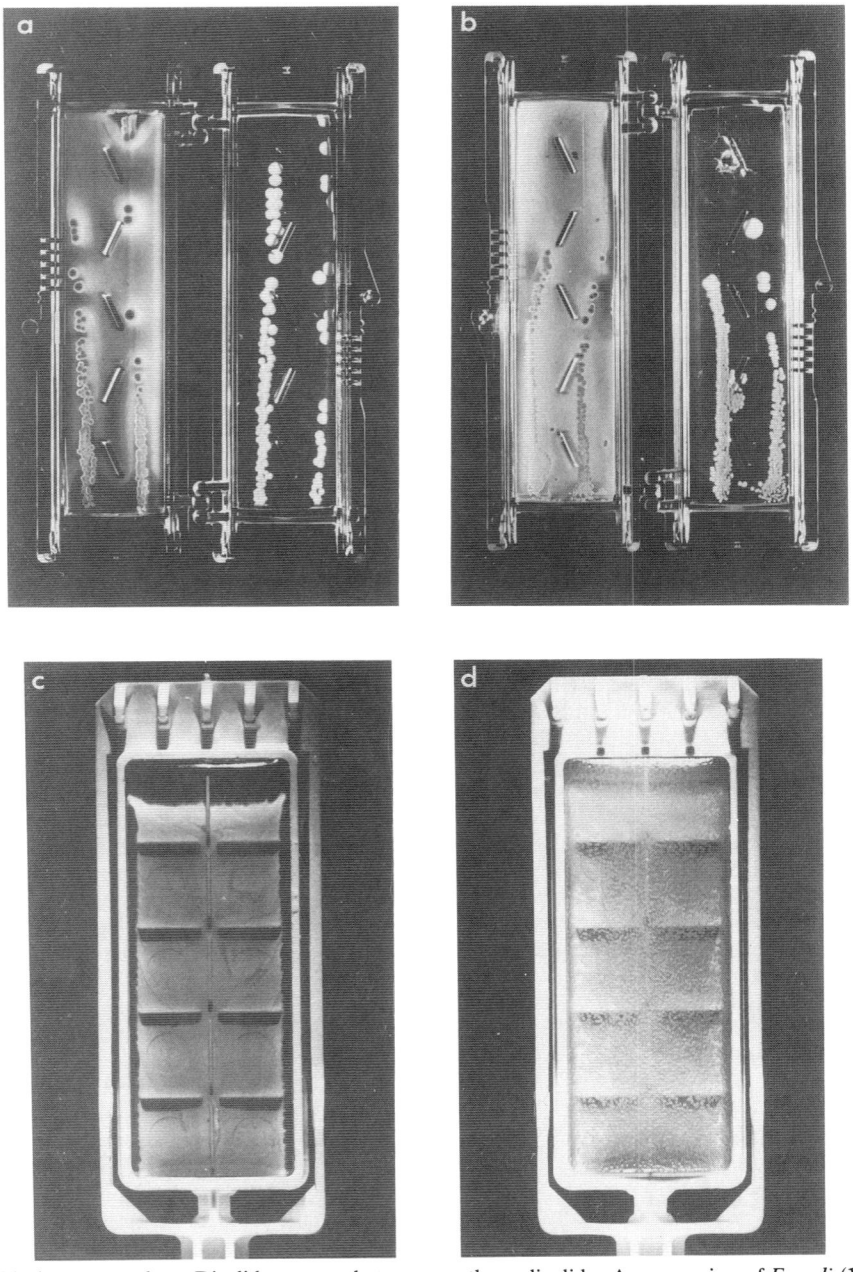

FIG. 4. Effect of antibiotic on growth on Diaslide versus that on growth on dipslide. A suspension of *E. coli* (10^5 CFU/ml) gave normal results on Diaslides whether or not ampicillin was present (a and b, respectively). However, no growth on the dipslide which was immersed in antibiotic-containing urine (c) was observed, compared with that on the control dipslide, which was dipped into the same suspension without added antibiotic (d).

carried out with urine samples which were obtained at random from 700 patients. Thirty percent of the samples were from geriatric and chronically ill hospitalized patients, and 70% of the samples were from other hospital wards and outpatient clinics. Urine samples were prescreened for catalase activity (Uriscreen; Diatech Diagnostica, Ltd., Rehovot, Israel) (3) in order to increase the proportion of positive cultures tested. Catalase-positive urine samples (n = 473) were then evaluated by three techniques: Diaslide, standard dipslide, and conventional culture. Diaslides (Diatech Diagnostica Ltd.) and dipslides (Uricult; Orion Diagnostica, Espoo, Finland) were used and interpreted according to the manufacturer's instructions and reference charts. Conventional culture was carried out with MacConkey and blood agar plates prepared with BBL culture media (Eldantech, Jerusalem, Israel) by using 10-μl disposable loops (Quad-Loops; Miniplast Ein Shemer, Kibbutz Ein Shemer, Israel). Cultures were incubated at 37°C in ambient air for 24 h.

Microbial identification and enumeration were performed with colonies isolated from agar plates. Identification of colonies was carried out by standard methods (2). In cases of mixed growth, the two major colony types were identified to the species level and the combined number of CFU per milliliter was used for the analyses.

RESULTS AND DISCUSSION

In order to compare colony numbers on a Diaslide with the cell density of a sample, urine was seeded with *E. coli* cells at concentrations which enabled enumeration of the number of colonies on the Diaslide. A linear correlation between Diaslide counts and plate counts at levels above 10^3 CFU/ml was observed (Fig. 2). As with ordinary dipslides, semiquantitative estimations of microbial levels could be carried out by comparison with those of reference Diaslides (Fig. 3).

An initial clinical evaluation in which Diaslide results were compared with those of conventional dipslides and plate cultures was conducted. Among the 473 cultures assayed, 243 (51%) were positive at the $\geq 10^4$ level and 212 (45%) were positive at the $\geq 10^5$ level (conventional culture). *E. coli* strains (n = 108), coagulase-negative staphylococci (n = 113), *Proteus* spp. (n = 83), *Klebsiella* spp. (n = 59), *Pseudomonas* spp. (n = 52), *Enterococcus faecalis* (n = 50), *Candida* spp. (n = 36), diphtheroids (n = 33), lactobacilli (n = 16), *Acinetobacter* spp. (n = 15), coagulase-positive staphylococci (n = 12), *Serratia* spp. (n = 7), *Enterobacter* spp. (n = 6), *Citrobacter* spp. (n = 4), beta-hemolytic streptococci (n = 4), and *Salmonella* spp. (n = 1) were among the 599 strains isolated from the positive Diaslides. The ability of the catalase prescreening test to identify *Enterococcus* infections was attributed to the presence of leukocytes in such specimens (3).

Results of the Diaslide versus those of standard plate cultures for cutoffs of 10^4 and 10^5 CFU/ml are shown in Table 1. Results of the Diaslide versus those of conventional dipslides are shown in Table 2. Table 3 presents a comparison of the sensitivities, specificities, positive predictive values, and total agreement of the various techniques. With the exception of a single category (86% percent total agreement between Diaslide and culture at the 10^4 cutoff, mainly due to 43 doubtful results for culture which scored as Diaslide negatives), Diaslide gave results comparable to those of both conventional culture and the dipslide technique.

In the present study, prescreened urine samples were employed in order to increase the proportion of positive cultures. This approach appears valid, since negative cultures were correctly identified with a high rate of success by using the Diaslide, with specificities ranging from 97.5 to 99.6% (Table 3). Nevertheless, future studies using nonscreened urine samples should also be performed.

Several potential advantages of the Diaslide became evident during the clinical evaluation. (i) Isolation of individual colonies was superior with the Diaslide, as only 5.5% of the Diaslides required subculturing, compared with 14.7 and 9.4% for the dipslide and plating techniques, respectively ($P < 0.001$ and $P < 0.03$, respectively, by the McNemar test). (ii) The Diaslide was much more convenient than conventional dipslides for sampling low volumes of urine (e.g., ca. 2 to 10 ml). (iii) Whereas dipslides had to be unscrewed following incubation for growth to be observed (because of condensation on the walls of the casing), microbial growth on Diaslide samples could be determined without removing the latter from the incubation stand. (iv) In general, growth on Diaslides was much more easily recognized than growth on dipslides because of the contrast between the isolation tracks and the surrounding medium, readily visible through the transparent casing. This was most evident with high concentrations of nonfermentative microorganisms which form transparent confluent films on conventional dipslides but are readily observed on Diaslides. (v) Since growth on the Diaslide is concentrated along two well-defined streaking lines, spurious exogenous contaminants were easy to distinguish.

One additional potential advantage of the Diaslide is the elimination of false negatives due to carryover of antibacterial agents from the urine (e.g., antibiotics) onto the agar surface. In order to illustrate this point, urine seeded with bacteria in the presence of ampicillin was sampled with the Diaslide and a dipslide (Fig. 4). Whereas the presence of ampicillin barely affected growth on the Diaslide, microbial growth on the dipslide was completely inhibited. Further experiments are under way to test the potential clinical relevance of this observation.

These data suggest that the Diaslide is a simple-to-use microbial-sampling device which incorporates the main advantages of conventional cultures and dipslides without compromising clinical performance. Whereas the Diaslide is currently being developed for urine testing, experiments are under way to examine its ability to sample other liquids, feces, and foods.

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